Historic, archived document

Do not assume content reflects current scientific knowledge, policies, or practices.

UNITED STATES DEPARTMENT OF AGRICULTURE

BULLETIN No. 739

Contribution from the Bureau of Animal Industry
JOHN R. MOHLER, Chief

Washington, D. C. PROFESSIONAL PAPER December 30, 1918

THE SIGNIFICANCE OF THE COLON COUNT IN RAW MILK

By

S. HENRY AYERS and PAUL W. CLEMMER
Bacteriologists, Dairy Division

CONTENTS

	Page
Present status of the question	1
Do organisms of the colon-aerogenes group indicate the presence of manure in fresh milk?	3
Can milk be produced commercially without contamination by organisms of the colon-aerogenes group?	9
How many organisms of the colon-aerogenes group can be introduced into milk during milking?	12
Why are high colon counts often found in raw milk?	19
Are both the B. coli and B. aerogenes types present in milk and if so is their relative proportion of any value?	27
Has the colon count any significance in raw milk?	31
Significance in fresh milk	31
Significance in milk that has been held	31
Summary	32
Literature cited	33

WASHINGTON
GOVERNMENT PRINTING OFFICE
1918

UNITED STATES DEPARTMENT OF AGRICULTURE

BULLETIN No. 739

Contribution from the Bureau of Animal Industry
JOHN R. MOHLER, Chief

Washington, D. C. PROFESSIONAL PAPER December 30, 1918

THE SIGNIFICANCE OF THE COLON COUNT IN RAW MILK.

By S. Henry Ayers and Paul W. Clemmer, *Bacteriologists, Dairy Division.*

CONTENTS.

	Page.
Present status of the question	1
Do organisms of the colon-aerogenes group indicate the presence of manure in fresh milk?	3
Can milk be produced commercially without contamination by organisms of the colon-aerogenes group?	9
How many organisms of the colon-aerogenes group can be introduced into milk during milking?	12
Why are high colon counts often found in raw milk?	19
Are both the B. coli and B. aerogenes types present in milk; and if so, is their relative proportion of any value?	27
Has the colon count any significance in raw milk?	31
Significance in fresh milk	31
Significance in milk that has been held	31
Summary	32
Literature cited	33

PRESENT STATUS OF THE QUESTION.

For many years the organisms of the colon-aerogenes group have occupied a position of very great importance in the eyes of sanitarians. From the public-health standpoint the quality of water supplies has been determined by means of the number of these organisms present; not because they are considered pathogenic but rather because they are present in fecal material in large numbers, and therefore their presence in any considerable number in water may indicate the presence of pathogenic organisms of fecal origin.

Investigations have shown, however, that the colon bacillus is by no means confined to the human intestines, but is often the prevailing form in the intestines of many animals, including cows. It would seem at first thought, therefore, that the presence of colon organisms in milk might afford a valuable means for determining pollution by cow feces. In fact, the determination of colon bacilli in milk as a means of indicating manurial pollution has become quite general on account of the value of the colon test as an index of the purity of water and the apparent analogy between the contamination of water by fecal material and the contamination of milk by cow feces.

75649°—18—Bull. 739——1

In this connection Savage (18) [1] states:

Lactose fermenters of the colon type are absent from pure milk drawn without contamination, and as far as it is known at present, all such organisms indicate outside pollution of the milk and in general (directly or indirectly) manurial pollution.

Kinyoun and Dieter (10) stated that their experience led them to conclude that the colon and streptococcus content of milk could be taken with reasonable certainty to indicate the amount of filth and dirt in milk. These authors also realized that temperature had an effect upon the colon content of milk, and in this connection they state:

It is evident from this fact alone that it will account for a considerable proportion of the number, but it must be understood that we do not believe that this factor alone is responsible for the enormous numbers found.

Again, Kinyoun (9) writes as follows:

What is the significance of the colon group in milk? The colon group comes from the intestinal canal of man and animals, indirectly may be found in soil, water, and food. This is such a well-established fact that it does not need discussion. When it gains access to food and drink it must be from one of these sources. Water frequently contains colon bacilli, and sanitarians agree that in determining the wholesomeness of water for drinking purposes the colon bacillus is the best index for purity.

Kinyoun while admitting that temperature played a part in the colon count believed that most of them gained entrance to the milk during milking, for in the same article he states:

When milk is contaminated by the colon group it indicates that it owes its presence there to uncleanly methods at the time of milking, or to the collection of the milk in dirty utensils, or to the contamination from air. The greater proportion of the colon bacilli gains entrance to the milk at the time of milking, and they owe their presence directly to fecal matter which has gained access to the pail by dirty, filthy cows, equally so the hands of the milker.

Other investigators are inclined to credit more importance to the growth of colon organisms in milk which has been held at high temperatures. Thus Harrison, Savage, and Sadler (4) made colon counts on a large number of samples in a study of the milk supply of Montreal, and, in speaking of the colon and aerogenes groups, state:

We regard any member of these groups as indicating manurial impurities in milk. A large number of this group present in the sample shows either carelessness and uncleanliness in milking, subsequent keeping at a high temperature, or, as most frequently happens, a combination of both.

In view of the fact that milk is an ideal medium for the growth of bacteria it seems that a determination of the colon bacilli as a means of indicating original contamination must be greatly complicated by the ability of these organisms to grow in the milk unless it is kept cold. Kendall, Day, and Walker (8) realized this difficulty and state:

The numbers of colon bacilli which may be present in a sample of milk do not necessarily furnish any evidence of the nature or extent of fecal contamination, for there is no way of distinguishing between the initial number of these organisms in a given sample of milk and their descendants.

[1] Figures in parentheses refer to "Literature cited" at end of paper.

If the colon count were a direct measure of original contamination during milking, it would be a most valuable aid in determining the purity of a milk supply. Can the colon count, however, be considered a direct measure of such contamination? This is a question which seems to be unsettled. As has been shown there are opinions on both sides of the question. While the test was formerly considered of considerable importance, more recently it has lost some of its significance because of the recognized ability of organisms of the colon and aerogenes group to grow rapidly in milk when not properly cooled and kept cold.

A test which gives promise of indicating original contamination, particularly fecal contamination, is worthy of attention, and it has seemed desirable to study this subject under controlled conditions with a view of determining the true value of the colon count in milk.

Throughout this paper the expressions "colon-aerogenes group" and "colon count" are used. The "colon count" means the number of organisms belonging to the colon-aerogenes group, and the term "colon-aerogenes group" as used includes both the *Bacillus coli* and *B. aerogenes* types of organisms. When any differentiation is made between the *B. coli* and *B. aerogenes* types it will be indicated in the text.

DO ORGANISMS OF THE COLON-AEROGENES GROUP INDICATE THE PRESENCE OF MANURE IN FRESH MILK?

If the organisms of the colon-aerogenes group are to be considered evidence of external contamination it must first be known whether or not they are present in the udders of cows. Both Savage (17) and Race (13) believe that organisms of this group are derived from sources outside the udder. Our results confirm this opinion, for in an examination of four cows no organisms of the colon-aerogenes group were ever found in milk drawn directly from the teats into sterile tubes. While only a few cows were examined, the results obtained, together with those of other investigators, seem to indicate that in general organisms of the colon-aerogenes group are not derived from the udder of the cow and therefore represent external contamination.

Raw market milk contains both the *Bacillus coli* and *B. aerogenes* types of organisms, as has been shown by the work of Rogers, Clark, and Evans (14), who found the colon-aerogenes group in such milk to be made up of nearly equal proportions of *B. coli* and *B. aerogenes* types. This being the case, it becomes necessary to know the source or sources of the *B. coli* and *B. aerogenes* types before they can be accepted as an indication of manurial contamination. It has been known for a long time that cow manure contains large numbers of organisms of the colon-aerogenes group, and the authors last men

tioned have shown that practically all the organisms found in this group are of the *B. coli* type. Among 150 cultures isolated from 16 samples of bovine feces they found only one culture of *B. aerogenes*. Even special methods used to demonstrate the presence of the *B. aerogenes* type in bovine feces failed to reveal their presence.

Our results in a general way confirm those of Rogers and his associates, for among 1,160 cultures isolated from 20 samples of fresh cow feces only 4 cultures of the *B. aerogenes* type were found. By means of special enrichment methods, however, it was often possible to isolate *B. aerogenes* types from 1/100 gram of fresh feces. The most satisfactory method of enrichment was to inoculate varying small amounts of cow feces into sterile skim milk. It seems evident, therefore, that the colon-aerogenes group found in cow feces consists mainly of the *B. coli* type. The *B. aerogenes* type is probably always present, but in very small numbers.

In view of the facts just presented, the contamination of milk by the colon-aerogenes group through cow feces would introduce principally organisms of the *B. coli* type. Occasionally the examination of fresh milk produced under clean conditions with sterilized utensils shows the presence of the colon-aerogenes group in small numbers which are mostly of the *B. aerogenes* type. Milk produced under these conditions would probably have an exceedingly small amount of fecal contamination, and taking into account the very small number of the *B. aerogenes* type in fresh cow feces compared to the number of the *B. coli* type, it is evident that the *B. aerogenes* found in the milk did not represent fecal contamination.

The *B. aerogenes* type being found at times in small numbers in low-count fresh milk led to the suspicion that the proportion of the *B. coli* and *B. aerogenes* types in cow feces might change as the feces aged and dried. Should this change occur it might explain the preponderance of the *B. aerogenes* type sometimes found in such milk. This seemed a very logical possibility because fecal material entering milk is probably not fresh but more or less dry, and gains entrance to the milk largely by dropping from the body of the cow. In order to determine whether the proportion of *B. coli* to *B. aerogenes* types changed in feces as it dried, a composite sample consisting of equal amounts of fresh feces from 4 cows was placed in a sterilized dish and allowed to dry at 86° F. (30° C.) to a practically constant moisture content, which after 14 days was 5 per cent. At regular intervals 1/100 of a gram of the material was inoculated into sterile milk. Two flasks of milk were inoculated with equal amounts of feces, one was incubated for 24 hours at 70° F. (21.1° C.) and the other for the same length of time at 86° F. (30° C.). After incubation, samples of milk

were plated on litmus-lactose-asparagin agar of the following composition:

	Per cent.
Agar	1.5
Asparagin	0.3
Lactose	1.0
Sodium dibasic phosphate	0.1
Saturated neutral solution of litmus	2.0

From these plates typical colonies of the colon-aerogenes group were then picked and inoculated into the dextrose-peptone broth of Clark and Lubs [3], and the methyl-red test made after five days as proposed by the same authors. By means of this test organisms are divided into *B. coli* and *B. aerogenes* types. If the proportion of the types changed as the feces dried, then enrichment in milk should show the presence of the *B. aerogenes* type. It is evident from Table 1, where the percentage of the two types after enrichment is shown, that the general proportion of *B. coli* to *B. aerogenes* which exists in fresh feces is not changed to any great extent as the feces dries. It is interesting to note, however, that when the feces was seven days old the enrichment method showed the presence of as high as 11 per cent of the *B. aerogenes* type. They were still present on the tenth day but not on the fourteenth, in milk held both at 70° F. (21.1° C.) and at 86° F. (30° C.). This indicates that as feces dries the proportion of *B. aerogenes* to *B. coli* in it may increase slightly, but, generally speaking, it is not believed that this is the main source of the *B. aerogenes* type.

TABLE 1.—*Effect of drying cow feces on the proportion of Bacillus coli and B. aerogenes types.*

Number of days dried at 70° F. (21.1° C.).	Held 24 hours at 70° F. (21.1° C.).			Held 24 hours at 86° F. (30° C.).		
	Colon count.	*B. coli.*	*B. aerogenes.*	Colon count.	*B. coli.*	*B. aerogenes.*
	Per c. c.	*Per cent.*	*Per cent.*	*Per c. c.*	*Per cent.*	*Per cent.*
0 (fresh)	320,000	100	0	26,000,000	100	0
1	2,500,000	100	0	23,000,000	100	0
2	420,000	100	0	103,000,000	100	0
3	760,000	100	0	210,000,000	100	0
6	490,000	100	0	280,000,000	100	0
7	680,000	89	11	132,000,000	99	1
10	6,500,000	99	1	270,000,000	96	4
14	6,500,000	100	0	37,000,000	100	0

The colon-aerogenes group of organisms is introduced into milk not only through feces but also from unsterilized utensils. In fact this is a very important source of contamination. Both *B. coli* and *B. aerogenes* types can be introduced into milk from unsterilized milk utensils, as may be seen in Table 2. The results given in the table represent a very extreme condition of production, such as

would rarely be met with under commercial conditions. The milk was produced in a dirty barn and placed in utensils handled in the following manner: The milk was poured out of the utensils promptly after milking, but the drippings were not removed till 24 hours later, when the utensils were washed in lukewarm water without the use of either brush or washing powder. During the time that the utensils were allowed to remain unwashed they were exposed to an atmospheric temperature averaging 85° F. (29.5° C.) Reference to Table 2 shows that in the six samples examined both the total and colon counts were very high in the fresh milk, and that most of the organisms of the colon-aerogenes group were of the *B. aerogenes* type, although a considerable percentage consisted of the *B. coli* type. These figures merely show that both types of organisms may be introduced through unsterilized utensils, but it must be clearly understood that the high total and the high colon counts obtained under this abnormal condition are very extreme, as will be subsequently seen. Milk may become heavily contaminated in this manner with both the *B. coli* and *B. aerogenes* types.

TABLE 2.—*Bacillus coli and B. aerogenes contamination due to filthy utensils.*

Sample No.	Total count.	Colon count.	*B. Coli.*	*B. aerogenes.*
	Per c. c.	*Per c. c.*	*Per cent.*	*Per cent.*
1	18,900,000	5,200,000	31	69
2	25,300,000	3,600,000	35	65
3	16,500,000	1,190,000	38	62
4	28,200,000	6,700,000	8	92
5	33,000,000	1,620,000	2	98
6	17,200,000	340,000	14	86

Organisms of the colon-aerogenes group may also be introduced into milk through the air. In this connection it is necessary to make some distinction between contamination by relatively large particles of feces which drop directly into milk from the body of the cow and very fine particles of dry feces and feed carried by the air in the form of dust. Under average conditions it is practically impossible to eliminate this kind of dust. An examination made of the air at various places as shown in Table 3 shows the nature of such dust infection. Twenty liters of air from each location was drawn through 20 cubic centimeters of sterile milk. A determination of the colon-aerogenes group was then made immediately by plating the milk on litmus-lactose-asparagin agar, 1/100 of a cubic centimeter being the lowest dilution made. It may be seen that in the fresh milk only two samples showed the presence of the colon-aerogenes group, and both were of the *B. aerogenes* type. The milk was examined again after 24 hours at 70° F. (21.1° C.), when all but 5 out of 22 samples showed the presence of organisms of the colon-aerogenes group.

TABLE 3.—*Nature of colon-aerogenes contamination from the atmosphere (20 liters of air drawn through 20 cubic centimeters of sterile milk).*

Location of air.	Examination of milk.					
	Fresh.			After 24 hours at 70° F. (21.1° C.).		
	Colon count.[1]	*B. coli.*	*B. aerogenes.*	Colon count.[1]	*B. coli.*	*B. aerogenes.*
		Per cent.	*Per cent.*		*Per cent.*	*Per cent.*
Barn air during feeding	0	0	0	36,000	91	9
Do	0	0	0	140,000	93	7
Barn air during currying	0	0	0	18,000,000	98	2
Barn air during feeding	0	.....	.....	0	.....	.....
Do	0	.....	.....	0	.....	.....
Barn air during currying	0	.....	.....	5,000	0	100
Barn air during milking	0	0	0	4,000,000	92	8
Barn air during currying	0	.....	.....	1,700,000	100	0
Barn air during milking	0	.....	.....	27,000	0	100
Do	0	.....	.....	0	.....	.....
Do	0	.....	.....	230,000,000	0	100
Do	0	.....	.....	205,000,000	0	100
Do	0	.....	.....	680,000	0	100
Barn air during currying	0	.....	.....	230,000	83	17
Do	0	.....	.....	12,800,000	0	100
Feed-room air	0	.....	.....	12,700,000	8	92
Do	0	.....	.....	0	.....	.....
Do	2,100	0	100	14,000,000	0	100
Outside air near barn	0	.....	.....	0	.....	.....
Do	0	.....	.....	6,700,000	0	100
Do	200	0	100	7,500,000	0	100
Do	0	.....	.....	60,000	0	100

[1] 0 means none in 1/100 of a cubic centimeter of milk.

It is of further interest to note that after enrichment in sterile milk the majority of samples contained organisms mostly of the *B. aerogenes* type, although in a few there was a large proportion of the *B. coli* type. It is not surprising that organisms of the *B. coli* type can be found in barn air, for they are present in large numbers in fresh cow feces, and probably actually multiply as the feces become dry. Evidence of this is furnished in Table 4, in which a composite sample of feces from 4 cows was examined when fresh, and again after 2, 10, and 14 days. The colon count is given per gram on both the dry and the wet basis. The calculations of the count on a dry basis show that there was an increase of from approximately 600,000 per gram when fresh to approximately 93,000,000 per gram when air-dried for 14 days. At the end of that time the organisms were still sufficiently active to grow readily on litmus-lactose-asparagin agar. It is easy to understand, therefore, how milk produced under clean conditions may become contaminated with organisms of the *B. coli* type. Contamination by this means, however, is not serious from the standpoint of numbers introduced, because the small numbers that enter from the air are greatly diluted in the milk.

TABLE 4.—*Direct increase of colon count in dried cow feces.*

Number of days dried at 70° F. (21.1° C.).	Moisture.	Colon count, wet.	Colon count, dry.
	Per cent.	*Per gram.*	*Per gram.*
0 (fresh)	84.5	320,000	590,000
2	79.0	1,500,000	2,685,000
10	6.3	49,000,000	51,940,000
14	5.0	89,000,000	93,450,000

It is not difficult to explain the presence of bacteria of the *aerogenes* type in air either inside or outside the barn, for they appear to be widely distributed on grains and other feeds, and in the soil. Rogers, Clark, and Evans (15) in their study of 166 organisms of the colon-aerogenes group occurring on grains, found that 151 were of the *B. aerogenes* type, while 8 resembled but were not identical with the *B. coli* type. An examination of many samples of alfalfa, cane, corn, and kafir-corn forage by Hunter (5) at the time of filling the silo showed *coli*-like cultures ranging in number from 1,000 to 1,000,000 per gram. A study of 95 of the organisms isolated from different kinds of silage, and of 15 strains obtained from growing fields of alfalfa and kafir corn showed about 21 per cent to be of the *B. coli* type and 79 per cent of the *B. aerogenes* type, as differentiated by the methyl-red test.

That *coli*-like organisms are present in soil has been shown by Johnson and Levine (7), who found them more prevalent in soils upon which crops were growing than in absolutely fallow areas receiving soil treatment. Aerogenes-cloacæ types were found to be the predominant *coli*-like forms in the soil.

It is apparent that the *B. aerogenes* type found in air may be derived from various sources, and it is not inconceivable that the organisms of this type found in market milk produced under clean conditions are the descendants of those introduced into the fresh milk in extremely small numbers by means of air contamination. It is also possible to contaminate milk with organisms of the *B. aerogenes* type by using unsterilized utensils.

Having surveyed the various means by which fresh milk is infected with organisms of the colon-aerogenes group it is now possible to answer the question "Do organisms of the colon-aerogenes group indicate the presence of manure in fresh milk?" The presence of the *B. coli* type indicates manurial contamination either directly from fecal material which drops into the milk principally from the body of the cow or indirectly through unsterilized utensils. It must be pointed out that the contamination by cow feces represents a much more serious form than that from unsterilized utensils. This statement may be explained by the fact that the introduction of cow feces into milk may carry organisms of bovine tuberculosis. Schroeder (19) has shown that cattle having tuberculosis swallow their sputum, and the tubercle bacilli in it pass through their bodies and out into the feces. The contamination of milk through unsterilized utensils represents a condition of careless handling in which there has been growth of the *B. coli* type in the utensils. While this kind of contamination may add as large a number of the *B. coli* type to milk as the fecal, it would not represent the same possibilities for infection with the bovine tubercle bacillus as does the latter. So far as the

B. coli type is concerned except for infection from manure and utensils, all other avenues of infection are probably of a negligible nature.

The *B. aerogenes* type does not indicate direct fecal contamination, because it is found only occasionally in manure and then only in an extremely small proportion in comparison with the *B. coli* type. On the other hand, the presence of the *B. aerogenes* type may indicate indirect fecal contamination through unsterilized utensils. As has been previously shown, this type of organism may be introduced through unsterilized utensils which have been held for 24 hours before washing in lukewarm water without a brush. Under this condition large numbers of the colon-aerogenes group were introduced which were principally of the *B. aerogenes* type. This can be explained only by the growth of organisms of the colon-aerogenes group in the utensils during storage following the original infection with the *B. aerogenes* type, which in all probability was due to either fecal or air contamination. If due to air contamination, the *B. aerogenes* type was very likely of nonfecal origin. The abnormality of this condition of production and the lack of sufficient results under normal conditions prevent a statement as to the probability of the contamination of milk by the *B. aerogenes* type through unsterile utensils. Air-borne infection probably plays a very important part in the introduction of this type in small numbers into milk, such contamination originating mainly in various feeds and in the soil. It seems evident, therefore, that the *B. coli* type in fresh milk indicates fecal contamination either directly or indirectly, while the *B. aerogenes* type occasionally may indicate indirect fecal contamination, but is usually an index of nonfecal contamination.

Although the *B. coli* type of organism may be considered an index of fecal contamination in fresh milk, it must be remembered that the colon count as usually made and as considered in this paper includes both types and does not differentiate between them. Our results confirm the opinion of Rogers, Clark, and Evans (16), who state:

> It is difficult to avoid the conclusion that while the presence of fecal bacteria in milk may be determined with great certainty, the ordinary presumptive tests and even the usual confirmatory tests do not necessarily prove the contamination of the milk with fecal matter. In order, therefore, to prove definitely the presence of the fecal colon type special confirmatory tests must be used to determine their presence.

CAN MILK BE PRODUCED COMMERCIALLY WITHOUT CONTAMINATION BY ORGANISMS OF THE COLON-AEROGENES GROUP?

This is a very important question, for if milk can be produced and handled under clean conditions without contamination by organisms of the colon-aerogenes group the presence of these organisms at any time in the milk would prove that it had not been so produced and handled.

Investigators who have studied the question do not believe that milk can be produced even under the best conditions without contamination by organisms of the colon-aerogenes group. Prescott (12) states:

> We may take certified milk to be a milk product which is as free from filth as it can be obtained under practical conditions, and when I say practical conditions I mean by using extreme care. Even milk which is obtained with ultra refinement and which may have a very low bacterial count is almost certain to contain some colon bacilli.

According to the author two-thirds of 200 samples of certified milk examined showed colon bacilli in 1 cubic centimeter, and of the remaining one-third fully one-half showed them to be present in 5 cubic centimeters or less. Race (13) has the same opinion on the subject, for he says:

> Milk even when produced under the best conditions is never quite free from *B. coli*, but if reasonable precautions are taken this group should not be present in 25 cubic centimeter quantities of byre milk.

The results obtained in our work lead us to the same opinion, namely, that milk can not be produced commercially, even under the best of conditions, without contamination by organisms of the colon-aerogenes group. Throughout the work, unless otherwise stated, the colon count has been determined by plating methods, using either litmus-lactose-asparagin agar, the composition of which has been previously given, or Endo's medium prepared according to the method of Kinyoun and Dieter (11). The value of litmus-lactose-asparagin agar as a selective medium for use in the determination of organisms of the colon-aerogenes group in milk can not be too highly emphasized. Sometimes the count on this medium is higher than on Endo's and sometimes lower, but it is believed that in general it gives a more accurate colon count than the Endo medium. Ayers and Johnson (2) called attention to the value of the asparagin medium, and the results obtained from its use in the present investigations again confirm its value. As mentioned, however, both media have been used and the highest counts recorded so as to give the highest colon count.

In order to determine whether milk could be produced without contamination by organisms of the colon-aerogenes group, 16 samples of milk were examined which was produced in a clean barn, from clean cows, and handled in sterilized utensils. The milk had a low total count, ranging from 1,130 to 8,900 per cubic centimeter. As may be seen in Table 5, none of the samples contained any organisms of the colon-aerogenes group in 1/10 cubic centimeter amounts. However, when these samples were held for 24 hours at 70° F. (21.1° C.) the colon count ranged from 900 to 303,000 per cubic centimeter. Since these organisms were present after 24 hours' incubation, some must have been in the fresh milk.

TABLE 5.—*Colon count from milk produced under clean conditions.*

Sample No.	Total count.	Colon count.	
		Fresh.	After 24 hours at 70° F. (21.1° C.).
	Per c. c.	*Per 1/10 c. c.*	*Per c. c.*
1	6,200	0	12,000
2	6,200	0	5,000
3	8,900	0	16,000
4	5,100	0	9,000
5	3,900	0	125,000
6	5,400	0	51,000
7	3,600	0	24,000
8	2,200	0	900
9	6,500	0	3,300
10	4,600	0	88,000
11	6,300	0	6,000
12	3,300	0	54,000
13	4,700	0	35,000
14	1,130	0	303,000
15	2,270	0	291,000
16	5,300	0	83,000

Further examination was made of 14 samples of certified milk as shown in Table 6. According to this table only 7 of the 14 samples showed the presence of colon organisms in 1 cubic centimeter of fresh milk, as determined in lactose-broth tubes; but after 24 hours they were found in every sample. These results, together with those of other investigators, clearly indicate that milk as commercially produced under the most careful conditions is never entirely free from organisms of the colon-aerogenes group.

TABLE 6.—*Colon count from certified milk.*

Sample No.	Total count in milk as delivered.	Colon count.				
		Fresh.	Held 24 hours at—		Held 48 hours at—	
			60° F. (15.6° C.).	70° F. (21.1° C.).	60° F. (15.6° C.).	70° F. (21.1° C.).
	Per c. c.	*Per c. c.*	*Per c. c.*	*Per c. c.*	*Per c. c.*	*Per c. c.*
1	7,400	10	10	4,600	23,700	4,400,000
2	10,400	[1] 1	110	38,000	49,000	15,000,000
3	5,200	[1] 1	10	2,900	46,000	2,890,000
4	10,100	20	280	79,000	55,000	16,400,000
5	8,200	0	100	400	1,900	1,130,000
6	4,200	[1] 1	170	16,600	120,000	6,100,000
7	12,600	10	320	13,500	169,000	12,500,000
8	5,900	[1] 1	20	1,800	21,600	560,000
9	14,800	0	0	3,100	2,700	610,000
10	11,400	0	0	400	4,600	5,300,000
11	10,100	0	460	38,000	186,000	13,100,000
12	22,800	0	10	1,100	6,400	3,700,000
13	7,800	0	20	6,500	10,800	8,700,000
14	15,700	0	150	5,800	36,000	1,510,000
Average.	10,471	.....	119	15,121	52,336	6,564,286

[1] 1 means at least 1 per cubic centimeter.

HOW MANY ORGANISMS OF THE COLON-AEROGENES GROUP CAN BE INTRODUCED INTO MILK DURING MILKING?

In order to interpret the significance of the colon count it is necessary to know not only that organisms of the colon-aerogenes group are always introduced into milk, but also in what numbers under both normal and abnormal conditions. In determining the numbers, the colon count has been made on a great many samples produced under different conditions. The results of these examinations have been summarized and are presented in Table 7. They are discussed briefly at this point in order to emphasize the fact that under normal conditions small numbers of organisms of the colon-aerogenes group are found in fresh milk. The averages given for the colon-aerogenes group are based on those samples showing the presence of colon bacilli in 1/100 of a cubic centimeter. Included in the table are the results of the examination of 14 samples of certified milk, which were examined at the time of delivery to the consumer. Except for the certified milk all the data presented in the table are based on the examination of milk produced under controlled conditions from 4 cows kept in a small experimental barn. The condition of the barn and the cows was changed at times from clean to extremely dirty in order to suit the purpose of the experiments. The handling of the utensils was also varied for similar reasons.

TABLE 7.—*Summary of colon-aerogenes contamination under different conditions of milk production.*

Conditions of milk production.	Samples examined.	Colon-aerogenes organisms in 1/100 c. c.		Range of colon counts.	Average colon count.	Average total bacteria.
	Number.	*Number.*	*Per cent.*	*Per c. c.*	*Per c. c.*	*Per c. c.*
Certified milk as delivered	14	[1] 0	0	1–20	0	10,471
Clean barn, clean cows, sterilized utensils (A)	86	6	6.9	100–200	116	6,091
Dirty barn, dirty cows, utensils sterilized (B)	56	6	10.7	100–400	183	36,541
Dirty barn, dirty cows, utensils washed at once after milking, but not sterilized (C)	84	24	28.6	([2])	([3])	465,086
Dirty barn, dirty cows, utensils held for 8 hours before washing, and not sterilized (D)	28	20	71.4	100–1,800	750	1,299,000

[1] 7+ in 1 cubic centimeter.
[2] 23 samples, 100–2,000. 1 sample, 28,400.
[3] Average of 23 samples, 557. Average of 24 samples, 1,717.

The examination of 86 samples of milk produced in a clean barn, from clean cows, and with sterilized utensils, is shown in Table 7. The term "sterilized" signifies steamed for 20 to 30 minutes. That the milk produced under these conditions was of high quality from a bacterial standpoint is shown by the average total count of 6,091 per cubic centimeter. Only 6 samples of the 86, or 6.9 per cent, contained organisms of the colon-aerogenes group in 1/100 of a cubic centimeter. The number of these organisms in the 6 samples ranged from 100 to 200 and averaged 116 per cubic centimeter.

A study was also made of 56 samples produced under conditions in which the cows were very dirty and the barn was cleaned only once or twice a week. The utensils were sterilized so that they would play no part in the contamination, thus demonstrating the extent of contamination by the colon-aerogenes group which might take place in a filthy barn. Of the 56 samples of milk produced under these conditions only 6, or 10.7 per cent, showed organisms of the colon-aerogenes group in 1/100 of a cubic centimeter. The range of these organisms was from 100 to 400 and averaged 183 per cubic centimeter. The average total count of the 56 samples was 36,541 per cubic centimeter. This at first thought might seem low in view of the filthy conditions of production described. The work of Ayers, Cook, and Clemmer (1), however, has shown that large numbers of bacteria are not generally introduced through manurial filth even under extreme conditions, but come largely from unsterilized utensils.

An analysis of 84 samples of milk produced under the same dirty conditions as previously described but with utensils which were not sterilized showed that 24 samples, or 28.6 per cent, contained organisms of the colon-aerogenes group in 1/100 cubic centimeter. The utensils in this case were washed in hot water containing washing powder within one hour after milking, rinsed in clean, cold water, and allowed to stand inverted until the next milking. The colon count of the 24 samples which showed positive results in 1/100 of a cubic centimeter ranged from 100 to 28,400 per cubic centimeter. Only one sample ran over 2,000, that one being 28,400. Including this high count the average would be 1,717, while leaving out this one high count the average for the remaining 23 samples would be 557 per cubic centimeter. These figures indicate clearly that unsterilized utensils play a prominent part in contamination of milk by the colon-aerogenes group. It will be noted in Table 7 that in the milk produced in unsterilized utensils the colon count was almost three times higher and the total count also higher than in the milk produced in sterilized utensils. In both cases the barn was equally dirty.

The manner in which the utensils are cleaned plays a very important part in the contamination of milk by organisms of the colon-aerogenes group, which is clearly shown in the last line of Table 7. The milk was produced in the same dirty barn as just mentioned, but the drainings were allowed to remain in the utensils for 8 hours, after which they were washed in hot water containing washing powder. Of the 28 samples produced under this condition 20, or 71.4 per cent, showed colon bacilli in 1/100 of a cubic centimeter. They ranged from 100 to 1,800, averaging 750 per cubic centimeter, and were no higher than in the milk produced in the previous experiment, in which the utensils were washed immediately, but a much greater percentage showed organisms of the colon-aerogenes group than in the previous case.

It is believed that the results shown in Table 7 cover the analyses of fresh milk produced under the widest range of conditions generally met with in the commercial production of milk. The conditions of production ranged from clean barn and cows and sterilized utensils to extremely dirty conditions and unsterilized utensils not washed till 8 hours after milking. The colon counts produced under such circumstances should represent the maximum number of these organisms usually introduced into milk.

The results on this point are considered so important that they have been plotted in figure 1. The graph shows the percentage of samples in which the colon count was 100 per cubic centimeter or more, as well as the percentage with lower counts. The letters correspond to those in Table 7; therefore the conditions of production are not repeated. Within each column is shown the colon count of every sample having 100 or more per cubic centimeter. Column A represents milk of high quality from a bacterial standpoint, only a small percentage of the samples showing the presence of organisms of the colon-aerogenes group in 1/100 of a cubic centimeter. A number of these samples were examined for the presence of the colon-aerogenes group in $\frac{1}{10}$ of a cubic centimeter, but none were found. Much larger amounts of milk of this quality would probably have to be examined in order to obtain positive tests.

Column B represents milk produced under extremely dirty barn conditions with sterilized utensils. This is a condition which would rarely be encountered because a dairyman who kept his barn and cattle in as dirty a condition as they were in this experiment probably would not be the kind who would take the trouble to sterilize utensils. This condition is, nevertheless, one of interest because only about 10 per cent of the samples produced under it showed the presence of the colon-aerogenes group in 1/100 of a cubic centimeter.

Column C represents results obtained from milk produced under the same dirty conditions as previously described, but the utensils were not sterilized. Milk produced under this condition represents, in a bacterial sense, more nearly the average milk of poor quality, but even then the condition of the barn and cattle was worse than is usually found. The term "dirty conditions" merely presents to the reader a degree of filth, and therefore depends upon his own opinion. It is believed, however, that the reader will be convinced of the degree of filth under which the milk was produced by referring to Plates I and II, where sediment disks from 1 pint of milk handled in unsterilized small-top and open pails under the filthy conditions mentioned are shown. One must admit that the quantity of manure shown on most of the sediment disks is greater than that usually found in milk. The illustrations show, besides the total bacterial count, the colon count per cubic centimeter in samples where they

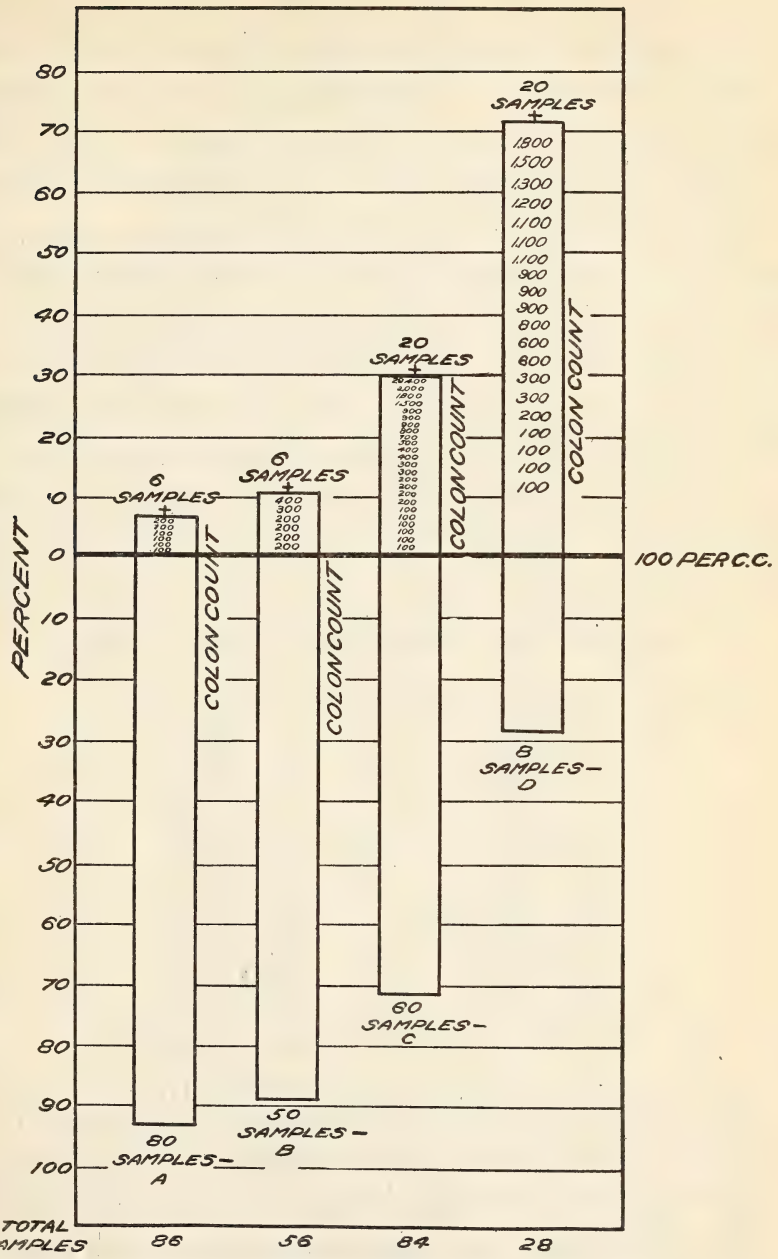


FIG. 1.—Colon-aerogenes group in fresh milk under different conditions of production (See Table 7 for conditions *A*, *B*, *C*, and *D*).

were present in $\frac{1}{100}$ of a cubic centimeter. Only 9 samples showed organisms of the colon-aerogenes group, and 8 of these ranged from 100 to 2,000, while the other had a count of 28,000 per cubic centimeter. Further study of the disks shows that there is no correlation between the quantity of manure and the colon count; some samples having a considerable amount of sediment showed no organism of the colon-aerogenes group, while others with a considerably less amount showed several hundred per cubic centimeter.

Reference to column C of figure 1 shows that about 72 per cent of the samples of milk produced under the conditions described did not contain any organisms of the colon-aerogenes group in $\frac{1}{100}$ of a cubic centimeter. Table 8 gives the results of a series of samples in which larger quantities of milk were examined. It is evident that milk, when fresh, even though produced under extremely dirty conditions and handled in unsterilized utensils, contains only a few organisms of the colon group. Of the 38 samples, 11 showed no colon organisms in $\frac{4}{10}$ of a cubic centimeter of milk, while in the remaining 27 samples the minimum colon count ranged from 3 to 75 per cubic centimeter.

TABLE 8.—*Small colon-aerogenes infection in fresh milk produced under dirty conditions.*

Sample No.	Total count.	Colon count.[1]	Sample No.	Total count.	Colon count.[1]
	Per c. c.	*Per c. c.*		*Per c. c.*	*Per. c. c.*
1	179,000	0	20	580,000	5
2	180,000	0	21	620,000	50
3	182,000	10	22	630,000	0
4	190,000	5	23	680,000	50
5	225,000	10	24	750,000	0
6	260,000	10	25	790,000	75
7	270,000	10	26	820,000	8
8	295,000	8	27	840,000	0
9	304,000	10	28	1,080,000	10
10	318,000	10	29	1,120,000	10
11	320,000	0	30	1,170,000	8
12	340,000	0	31	1,310,000	3
13	366,000	0	32	1,320,000	3
14	450,000	5	33	1,440,000	5
15	460,000	0	34	1,450,000	0
16	490,000	3	35	1,600,000	0
17	490,000	10	36	1,940,000	10
18	497,000	8	37	1,950,000	10
19	560,000	75	38	2,400,000	10

[1] Cipher indicates no organisms of the colon-aerogenes group in $\frac{1}{10}$ cubic centimeter of milk.

Additional sediment disks are shown in Plate III, the corresponding samples of which are also included in column D of figure 1. In this case the utensils were not washed till 8 hours after milking, but otherwise the conditions were the same as in the previous experiment. Here, again, even with very large quantities of manure found in the milk, together with the increased possibilities of colon contamination from the unsterilized utensils, the colon count never ran over 2,000 per cubic centimeter when the milk was fresh. Plates I, II, and III show that under these conditions there is no definite

relation between the quantity of manure and the colon count. This is undoubtedly due to the variable number of such organisms found in cow feces, and to the different degrees of such contamination introduced through unsterilized utensils.

The results thus far presented show very clearly that unsterilized utensils play a prominent part in the contamination of milk with organisms of the colon-aerogenes group. To emphasize this point an experiment was conducted in which the milk from three cows went into sterile utensils while that from three others went into unsterilized utensils which had been allowed to stand for 24 hours before washing. All six cows were clean and were kept in a clean barn, and the only chance for difference in the contamination of the milk was due to the unsterilized utensils. The results in Table 9 show that in the milk handled in sterilized utensils there were no organisms of the colon-aerogenes group present in 1/100 of a cubic centimeter, while all the samples of milk handled in unsterilized utensils showed the presence of these organisms, ranging from 1,100 to 350,000, and averaging 80,650 per cubic centimeter in the fresh milk. This, of course, represents contamination from unsterilized utensils which were handled in an unusual manner, but it serves to emphasize the importance of sterilized utensils in connection with the colon count.

TABLE 9.—*Effect of sterilized utensils on colon count.*

Sample No.	Sterilized utensils.		Utensils not sterilized and not washed for 24 hours.	
	Total count.	Colon count.[1]	Total count.	Colon count.
1	17,100	0	2,050,000	91,000
2	4,600	0	2,800,000	72,000
3	7,300	0	2,320,000	120,000
4	5,700	0	2,210,000	350,000
5	3,900	0	254,000	1,100
6	4,300	0	680,000	1,400
7	10,200	0	450,000	5,200
8	6,300	0	365,000	4,500
Average...	7,425	.....	1,391,125	80,650

[1] 0 indicates none in 1/100 of a cubic centimeter.

At this point it is possible to answer the question "How many organisms of the colon-aerogenes group can be introduced during milking?" The answer is, not over 2,000 per cubic centimeter, and is based on the examination of 254 samples produced under both clean and unusually filthy conditions, only one sample of which showed a colon count of over 2,000 per cubic centimeter in fresh milk. A summary of these counts is shown in Table 7 and also in figure 1. If this is the maximum number of organisms of the

colon-aerogenes group which can be introduced into milk under normal conditions of production, it will be of great importance in connection with the significance of the colon count. If correct, it means that any colon count over 2,000 per cubic centimeter could at once be attributed to growth.

The results obtained in our experiments indicate that, generally speaking, 2,000 per cubic centimeter is the maximum number of organisms of the colon-aerogenes group introduced into fresh milk. It is realized, however, that occasionally abnormal conditions may be encountered where the colon count in fresh milk runs higher than the maximum limit mentioned. The only time such a condition was met in the experiments occurred during the period from August 21 to September 12, 1917, when milk was produced under the dirty conditions as previously described. The utensils were held for 24 hours before washing, and then simply washed in lukewarm water without brush or washing powder. During this 24-hour period the air temperature ranged from 70° F. (21.1° C.) to 98° F. (36.7° C.), a very favorable condition for growth of organisms of the colon-aerogenes group in the unwashed utensils. Table 10 shows that the colon count of 12 samples of fresh milk produced under these conditions ranged from 80,000 to 6,700,000 per cubic centimeter. The experiment was stopped on September 12 and was repeated about October 1 under the same conditions with the exception that the air temperature averaged much lower, ranging between 40° F. (4.4° C.) and 70° F. (21.1° C.). It was found that at this time the colon counts were all within the maximum of 2,000 per cubic centimeter. This led to the belief that the holding of utensils containing milk drainings at the high temperatures which prevailed during the period from August 21 to September 12 was responsible for the extremely heavy contamination by organisms of the colon-aerogenes group.

In order to confirm this opinion further experiments were conducted during October under the same dirty-barn conditions except that the utensils, which were not washed for 24 hours, were held in artificially heated rooms. From the results in the lower part of Table 10 it is evident that even under these conditions it was impossible to obtain the high colon counts that had previously been obtained. While it was impossible to maintain as high temperatures artificially as occurred normally in our earlier experiment, temperatures as high as 86° F. (30° C.) were obtained, and it is believed that in many cases the utensils were held at as high temperature as in the previous experiment. In the last experiment where the utensils were held in artificially heated rooms the colon count ranged from 100 to 350,000 per cubic centimeter. Only 9 of the 25 samples showed more than 2,000 organisms of the colon-aerogenes group per cubic centimeter.

TABLE 10.—*Number of organisms of colon-aerogenes group introduced into fresh milk under abnormal conditions.*

Conditions.		Sample No.	Total count.	Colon count.
			Per c. c.	*Per c. c.*
Utensils held 24 hours before washing; not sterilized. August 21 to 30, and September 8 to 17; air temperature 70° F. to 98° F. (21.1° C. to 37.5° C.)		1	18,900,000	5,200,000
		2	25,300,000	3,600,000
		3	16,500,000	1,190,000
		4	22,700,000	5,100,000
		5	28,200,000	6,700,000
		6	33,000,000	1,620,000
		7	17,200,000	340,000
		8	33,500,000	690,000
		9	54,000,000	1,780,000
		10	65,000,000	2,350,000
		11	25,000,000	1,190,000
		12	2,040,000	80,000
Utensils held 24 hours before washing; not sterilized.	October 3 to 12, 75° F. (23.9° C.).	1	2,050,000	91,000
		2	2,800,000	72,000
		3	2,320,000	120,000
		4	2,210,000	350,000
		5	680,000	1,400
		6	450,000	5,200
		7	365,000	4,500
	October 22 to 25, 60° F. (21.1° C.).	1	1,010,000	(1)
		2	340,000	200
		3	690,000	100
		4	580,000	300
	October 22 to 25, 75° F. (23.9° C.).	1	270,000	1,400
		2	560,000	500
		3	370,000	300
		4	490,000	400
	October 29 to November 5, 60° F. (21.1° C.).	1	163,000	1,200
		2	490,000	2,200
		3	410,000	800
		4	288,000	700
		5	310,000	100
	October 29 to November 5, 86° F. (30° C.).	1	1,100,000	37,000
		2	225,000	13,000
		3	98,000	400
		4	161,000	300
		5	42,000	200

[1] 0 in 1/100 c. c.

These results indicate that under abnormal conditions the colon count may be over 2,000 per cubic centimeter in fresh milk, but it is believed, in view of our experiments, that such exceptional conditions are seldom encountered. These high counts therefore do not detract from the value of the 2,000 maximum colon count per cubic centimeter, which has been set as the maximum contamination by organisms of the colon-aerogenes group in fresh milk.

WHY ARE HIGH COLON COUNTS OFTEN FOUND IN RAW MILK?

Assuming the correctness of the fact just established that fresh milk produced under normal conditions very rarely contains over 2,000 organisms of the colon-aerogenes group per cubic centimeter, the question "Why are high colon counts often found in raw milk?" can be answered by the statement that high colon counts in raw milk are due to growth which takes place between the time of production and that of consumption.

If organisms of the colon-aerogenes group were not able to grow in milk the colon count would be of far greater value as an index of con-

tamination during production. Jackson (6) evidently believed that these organisms did not grow in milk, for he states:

> Contrary to expectation it has been found that under ordinary conditions, considerable numbers of other bacteria being present, *B. coli* does not increase in milk, or if so only to a very slight extent, then gradually dies out. On this account the amount of original contamination may be determined.

Most investigators, however, have recognized the fact that organisms of the colon-aerogenes group were able to multiply in milk and that this fact complicated the significance of the colon count. Kinyoun (9), while recognizing that the age of the milk and the temperature at which it has been held are important, believed that original contamination was responsible for the most part in causing high colon counts. According to Prescott (12), organisms of the colon group will grow in milk which is held at 50° F. (10° C.), and he found that even when milk was packed in ice for several days the rate of increase was much greater than had been expected. This author believes that the question of growth must be taken into consideration in interpreting the colon count, for he says:

> The mere question of the numbers of colon bacilli found in milk or cream may be settled or explained in two ways. It may have been due to initial infection or due to growth. Here discrimination must be employed as to the relative number of organisms and the types which are present in order to determine which of these explanations is the satisfactory one.

The opinion of Race (13) on this subject is expressed in the following statement:

> When milk is kept at a temperature not exceeding 45° F. (7.2° C.) the *B. coli* do not increase and this temperature may therefore be regarded as the critical anabolic temperature. Above this point they rapidly multiply and in summer the *B. coli* content of milk must be regarded as due more to reproduction than original contamination.

In the several pages following are shown the results of a large number of samples of milk produced under different barn conditions and the growth of organisms of the colon-aerogenes group at various temperatures. Of these, 20 samples were produced under clean conditions and handled in sterilized utensils, 34 samples were produced under dirty conditions with sterilized utensils, and 23 samples were produced under similar dirty conditions but handled in unsterilized utensils, and in each case the samples were held at 50° F. (10° C.) and duplicate samples were held at 60° F. (15.6° C.), and the colon count determined in the milk when fresh and after each succeeding 24 hours up to 96 hours. The results obtained when the milk was held at 50° F. (10° C.) are shown in Table 11. The results have been summarized and the per cent of samples showing the presence of organisms of the colon-aerogenes group in 1/100 of a

cubic centimeter of milk and their average counts are given, together with the per cent of samples which did not show the colon-aerogenes group in this quantity of milk. Where more than one sample of a group showed colon organisms the range is also given. The colon counts represent the highest number determined on either litmus-lactose-asparagin agar or on Endo medium.

TABLE 11.—*Growth and frequency of organisms of colon-aerogenes group at 50° F. (10° C.)*

Number of samples.	Conditions.	Average total count.	Colon count.	
			Fresh.	24 hours.
20	Barn clean, cows clean, utensils sterilized (A).	*Per c. c.* 4,245	*Per c. c.* 100 per cent— in 1/100 c. c.	*Per c. c.* 94.4 per cent—. 5.6 per cent+. Average colon count, 300.[1]
34	Barn dirty, cleaned once a week; cows dirty; utensils sterilized (B).	38,262	94.1 per cent—. 5.9 per cent+. Range, 100 to 300. Average colon count, 200.	100 per cent—.
23	Same as B, but utensils not sterilized.	161,083	95.6 per cent—. 4.4 per cent+. Average colon count, 400.	100 per cent—.

Number of samples.	Conditions.	Average total count.	Colon count.		
			48 hours.	72 hours.	96 hours.
20	Barn clean, cows clean, utensils sterilized (A).	*Per c. c.* 4,245	*Per c. c.* 94.4 per cent—. 5.6 per cent+. Average colon count, 3,200.	*Per c. c.* 83.3 per cent—. 16.7 per cent+. Range, 1,600 to 28,000. Average colon count, 10,533.	*Per c. c.* 83.3 per cent—. 16.7 per cent+. Range, 1,600 to 79,000. Average colon count, 27,533.
34	Barn dirty, cleaned once a week; cows dirty; utensils sterilized (B).	38,262	95.6 per cent—. 4.4 per cent+. Average colon count, 1,900.	95.6 per cent—. 4.4 per cent+. Average colon count, 3,200.	54.2 per cent—. 45.8 per cent+. Range, 2,000 to 186,000. Average colon count, 24,172.
23	Same as B, but utensils not sterilized.	161,083	94.7 per cent—. 5.3 per cent+. Average colon count, 11,600.	83.3 per cent—. 16.7 per cent+. Range, 4,500 to 12,500. Average colon count, 8,100.	55.5 per cent—. 44.5 per cent+. Range, 3,300 to 22,000. Average colon count, 13,300.

[1] Average colon count per cubic centimeter in samples showing them in $\frac{1}{100}$ of a cubic centimeter and over.

The average total count of 20 samples of milk produced under clean conditions was 4,245, indicating a high quality of milk, from a bacterial standpoint. No organisms of the colon-aerogenes group were found in 1/100 of a cubic centimeter in any of these samples when fresh. After 24 hours 94.4 per cent of the samples were still negative, while 5.6 per cent were positive. The same results were obtained at the end of 48 hours, only one sample showing organisms of

colon-aerogenes group, and this one contained 3,200. After the milk had been held 72 hours, 83.3 per cent were still negative in 1/100 cubic centimeters while 16.7 per cent were positive. The colon count ranged from 1,600 to 28,000, the average being 10,533 per cubic centimeter. After 96 hours at 50° F. (10° C.) the same percentage of the samples showed growth as after 72 hours, but the average colon count was higher.

Among the 34 samples of milk produced under dirty conditions and handled in sterilized utensils 5.9 per cent showed organisms of the colon-aerogenes group in 1/100 of a cubic centimeter of milk. The range in colon count was from 100 to 300 and the average 200 per cubic centimeter. After 24 hours at 50° F. (10° C.) none of the samples showed the organisms in 1/100 of a cubic centimeter. This is explained by the fact that the colon count in the samples which showed positive results in the fresh milk ranged from 100 to 300. Since 1/100 of a cubic centimeter of milk was plated this meant from one to three colonies per plate, and as there was probably no growth during the 24-hour holding period at 50° F. (10° C.), these organisms were missed in a hundredth dilution on the second day's plating. At the end of 72 hours only 4.4 per cent of the samples showed colon-aerogenes organisms, which averaged 3,200 per cubic centimeter. There was a slight growth among these samples between 72 and 96 hours, for, as may be noted in the table, 45.8 per cent showed positive results at the end of 96 hours. The colon counts ranged from 2,000 to 186,000 and averaged 24,172 per cubic centimeter. The results obtained in the examination of 23 samples produced under dirty conditions and handled in unsterilized utensils agreed very closely with those just discussed.

From these results it seems evident that there is practically no growth of organisms of the colon-aerogenes group in milk produced under normal conditions when it is held for a period of 48 hours at 50° F. (10° C.). After 72 hours at this temperature there was evidence of multiplication, which was still further increased by holding for 96 hours.

Duplicate samples of the milk produced under the conditions presented in Table 11 were held at 60° F. (15.6° C.) and the summarized results are given in Table 12. It will be noted that there is a marked difference in the growth of the organisms of the colon-aerogenes group in milk held at the higher temperature. Quite high colon counts were obtained in the majority of samples held at 60° F. (15.6° C.) even in 24 hours. No further discussion of the results is necessary, as the effect of temperature on the colon count is plainly shown by the results presented.

TABLE 12.—*Growth and frequency of organisms of colon-aerogenes group at 60° F. (15.6° C.).*

Number of samples.	Conditions.	Average total count.	Average colon count.	
			Fresh.	24 hours.
20	Barn clean, cows clean, utensils sterilized (A).	*Per c. c.* 4,245	*Per c. c.* 100 per cent — in 1/100 c. c.	*Per c. c.* 66.6 per cent —. 33.4 per cent +. Range, 400 to 76,000. Average colon count, 14,083.[1]
34	Barn dirty, cleaned once a week, cows dirty, utensils sterilized (B).	38,262	94.1 per cent —. 5.9 per cent +. Range, 100 to 300. Average colon count, 200.	31 per cent —. 69 per cent +. Range, 1,000 to 510,000. Average colon count, 79,175.
23	Same as B, but utensils not sterilized.	161,083	95.6 per cent —. 4.4 per cent +. Average colon count, 400.	33.3 per cent —. 66.7 per cent +. Range, 6,100 to 710,000. Average colon count, 156,450.

Number of samples.	Conditions.	Average total count.	Average colon count.		
			48 hours.	72 hours.	96 hours.
20	Barn clean, cows clean, utensils sterilized (A).	*Per c. c.* 4,245	*Per c. c.* 50 per cent —. 50 per cent +. Range, 1,000 to 840,000. Average colon count, 318,333.	*Per c. c.* 35.3 per cent —. 64.7 per cent +. Range, 1,700 to 25,500,000. Average colon count, 5,506,518.	*Per c. c.* 37.5 per cent —. 62.5 per cent +. Range, 4,000 to 152,000,000. Average colon count, 27,288,900.
34	Barn dirty, cleaned once a week, cows dirty, utensils sterilized (B).	38,262	4.0 per cent —. 96.0 per cent +. Range, 98,000 to 21,600,000. Average colon count, 3,285,750.	8.3 per cent —. 91.7 per cent +. Range, 900,000 to 68,000,000. Average colon count, 18,786,364.	100 per cent +. Range, 1,000,000, to 66,000,000. Average colon count, 23,008,333.
23	Same as B, but utensils not sterilized.	161,083	26.3 per cent —. 73.7 per cent +. Range, 84,000 to 4,300,000. Average colon count, 882,929.	5.5 per cent —. 94.5 per cent +. Range, 64,000 to 42,000,000. Average colon count, 4,579,117.	100 per cent +. Range, 115,000 to 168,000,000. Average colon count, 15,600,611.

[1] Average colon count per cubic centimeter in samples showing them in 1/100 of a cubic centimeter and over.

When milk is held at higher temperatures the growth of organisms of the colon-aerogenes group increases in rapidity, which is shown by the results given in Table 13. Twenty samples of milk produced under dirty conditions with unsterilized utensils were held at 50° F. (10° C.) and 70° F. (21° C.) and the colon count determined in the fresh milk and after holding for 24 hours. Although this milk was produced under extremely filthy conditions, only 3 of the samples when fresh showed organisms of the colon-aerogenes group in 1/100 of a cubic centimeter. This series contained the only sample of fresh milk produced under normal conditions which showed a colon count of over 2,000 per cubic centimeter. The results in this table

show the remarkable difference in growth of organisms of the colon-aerogenes group in milk held at 50° F. (10° C.), compared with 70° F. (21° C.). While there is some indication of slight growth in a few of the samples held at 50° F. (10° C.), there was the rapid multiplication of the organisms in milk held at 70° F. (21° C.), the average colon count in 20 samples held for 24 hours at this temperature being 7,385,500 per cubic centimeter.

TABLE 13.—*Growth of organisms of colon-aerogenes group at 50° F. (10° C.) and 70° F. (21.1° C.).*

Sample No.	Total count.	Colon count.[1]		
	Fresh.	Fresh.	Held for 24 hours at 50° F. (10° C.).	Held for 24 hours at 70° F. (21.1° C.).
1	225,000	0	100	180,000
2	1,140,000	28,400	83,000	55,500,000
3	270,000	0	200	2,600,000
4	680,000	0	0	2,350,000
5	1,080,000	0	0	3,400,000
6	490,000	0	800	5,100,000
7	850,000	900	1,300	8,300,000
8	840,000	0	200	2,700,000
9	1,450,000	0	600	2,520,000
10	2,400,000	0	0	12,800,000
11	1,950,000	0	0	22,000,000
12	260,000	0	0	1,250,000
13	610,000	2,000	600	15,300,000
14	340,000	0	0	830,000
15	630,000	0	0	1,170,000
16	179,000	0	0	1,180,000
17	366,000	0	0	6,400,000
18	304,000	0	0	120,000
19	460,000	0	0	1,810,000
20	620,000	0	400	2,200,000

[1] 0 indicates none in $\frac{1}{100}$ of a cubic centimeter.

Further studies on the growth of organisms of the colon-aerogenes group at different temperatures are shown in Table 14. Three different sets of conditions were used so as to determine the subsequent growth in three distinct grades of fresh milk. Special attention is called to the colon counts after the milk had been held for 6 hours at 86° F. (30° C.). Among the 22 samples of milk produced in the dirty barn and handled in sterilized utensils there was practically no increase in the colon count after 6 hours at 86° F. (30° C.), but after 24 hours at the same temperature the colon count had reached the high figure of 63,279,545 per cubic centimeter. Twenty-six samples produced in the dirty barn and handled in unsterilized utensils showed after 6 hours a distinct increase in the colon count in most of the samples, and after 24 hours the colon count averaged about 24,000,000 per cubic centimeter.

Similar increases occurred among the 20 samples produced in a dirty barn and handled in unsterilized utensils which had not been washed until 8 hours after milking. The average colon count of the fresh milk was 595 per cubic centimeter. After 6 hours, at 86° F. (30° C.) the average count had increased to 37,525, while after 24

hours at the same temperature the average count was about 205,000,000. The difference in the rate of growth of organisms of the colon-aerogenes group at this temperature seems to vary somewhat with the amount of initial contamination, as is shown by the colon counts of the fresh milk and after 6 and 24 hours. Further observation of Table 14 shows that when the milk was held at 50° F. (10° C.) there was little or no increase over the count in the fresh milk. There are, however, a few samples which show a distinct growth.

TABLE 14.—*Influence of conditions of production on the subsequent increase of organisms of the colon-aerogenes group.*

Sample No.	Conditions.	Total count in fresh milk.	Colon count.[1]			
			Fresh.	Held 6 hours at 86° F. (30° C.).	Held 24 hours at—	
					50° F. (10° C.).	86° F. (30° C.).
1	Barn dirty, cleaned twice a week; utensils sterilized.	27,000	0	200	0	290,000,000
2		58,000	100	400	0	294,000,000
3		54,000	100	0	100	126,000,000
4		83,000	0	100	0	11,900,000
5		28,000	0	0	0	550,000
6		96,000	0	0	0	14,200,000
7		11,800	0	0	0	38,000,000
8		39,000	0	100	0	2,400,000
9		21,600	0	0	0	1,700,000
10		12,400	0	0	0	5,300,000
11		10,200	0	100	0	139,000,000
12		22,000	0	0	0	11,500,000
13		12,500	0	0	0	1,100,000
14		42,000	100	0	0	250,000,000
15		16,400	0	[2]	0	6,300,000
16		22,800	0	[2]	0	42,000,000
17		24,000	0	[2]	0	12,400,000
18		37,000	0	[2]	0	17,600,000
19		13,500	0	[2]	300	8,600,000
20		57,000	0	[2]	0	54,000,000
21		14,200	400	[2]	100	37,000,000
22		15,500	0	[2]	0	28,600,000
Average		32,477				63,279,545
1	Barn dirty, cleaned twice a week; utensils washed immediately but not sterilized.	1,320,000	0	0	0	60,000,000
2		790,000	0	400	100	450,000,000
3		1,600,000	0	0	0	1,900,000
4		295,000	0	500	0	330,000,000
5		1,940,000	0	200	0	2,800,000
6		690,000	100	300	0	7,500,000
7		1,310,000	0	1,400	0	650,000
8		520,000	100	4,500	0	248,000,000
9		750,000	0	0	0	11,400,000
10		460,000	100	2,600	0	510,000,000
11		320,000	0	[2]	0	9,400,000
12		440,000	400	[2]	800	260,000,000
13		180,000	0	0	0	46,000,000
14		490,000	0	900	1,100	285,000,000
15		318,000	0	1,000	0	210,000,000
16		497,000	0	1,200	100	480,000,000
17		580,000	0	[2]	0	174,000,000
18		440,000	200	[2]	100	216,000,000
19		780,000	100	2,400	100	420,000,000
20		710,000	800	46,000	600	525,000,000
21		590,000	100	8,600	[2]	580,000,000
22		310,000	900	41,000	[2]	430,000,000
23		190,000	0	800	100	55,000,000
24		230,000	300	74,000	15,100	400,000,000
25		182,000	0	1,100	100	152,000,000
26		340,000	900	48,000	900	244,000,000
Average		625,846		10,677		234,948,077

[1] 0 indicates no organisms of the colon-aerogenes group in 1/100 of a cubic centimeter of milk.
[2] No determinations made.

TABLE 14.—*Influence of conditions of production on the subsequent increase of organisms of the colon-aerogenes group*—Continued.

Sample No.	Conditions.	Total count in fresh milk.	Colon count.			
			Fresh.	Held 6 hours at 86° F. (30° C.).	Held 24 hours at—	
					50° F. (10° C.).	86° F. (30° C.).
1	Barn dirty, cleaned twice a week; utensils washed after 8 hours but not sterilized.	670,000	100	200	0	260,000,000
2		820,000	0	2,100	100	117,000,000
3		3,300,000	1,200	35,200	1,400	342,000,000
4		1,800,000	100	3,100	200	240,000,000
5		1,170,000	0	400	0	181,000,000
6		1,440,000	0	700	200	159,000,000
7		620,000	100	[1]	300	1,850,000
8		860,000	300	[1]	500	194,000,000
9		570,000	800	1,500	[1]	120,000,000
10		630,000	300	9,700	[1]	162,000,000
11		1,300,000	600	1,400	800	72,000,000
12		2,720,000	1,800	510,000	3,300	285,000,000
13		1,120,000	0	600	0	96,000,000
14		1,840,000	900	14,200	1,200	330,000,000
15		1,080,000	1,100	4,500	1,500	460,000,000
16		1,920,000	1,300	4,300	2,700	370,000,000
17		1,160,000	100	100	1,300	160,000,000
18		3,820,000	1,100	12,400	21,000	196,000,000
19		2,200,000	600	[1]	[1]	115,000,000
20		4,300,000	1,500	[1]	[1]	220,000,000
Average		1,667,000	595	37,525	2,156	204,875,000

[1] No determinations made.

The results of these experiments in general confirm the opinion of Race (13) that organisms of the colon-aerogenes group do not grow at 45° F. (7.2° C.), for as a rule little or no growth was found to take place when milk was held at 50° F. (10° C.) for lengths of time comparable with commercial practice.

In studying the growth of organisms of the colon-aerogenes group in milk held at different temperatures, $\frac{1}{100}$ of a cubic centimeter was the largest quantity of milk examined, so that any growth occurring in quantities less than 100 per cubic centimeter would not have been detected. The results obtained therefore do not conflict with those of Prescott (12), who found that milk which showed no colon bacilli in a single cubic centimeter when fresh, after 24 hours at 50° F. (10° C.) contained from 10 to 50 per cubic centimeter.

When milk is kept at temperatures above 50° F. (10° C.) there is a rapid growth of organisms in the colon-aerogenes group in direct proportion to the temperature and time to which milk might be subjected under commercial conditions.

ARE BOTH THE B. COLI AND B. AEROGENES TYPES PRESENT IN MILK; AND IF SO, IS THEIR RELATIVE PROPORTION OF ANY VALUE?

Since Rogers and his associates (14, 15) have shown that organisms of the colon-aerogenes group found in bovine feces are principally of the *Bacillus coli* type while those occurring on grains correspond to the *B. aerogenes* type, it seems as if a differentiation of these types in milk might be of value in determining the source of contamination. Raw milk ordinarily contains both the *B. coli* and the *B. aerogenes* type. Rogers and his associates found that nearly 50 per cent of the cultures isolated from milk were of the *B. aerogenes* type.

In our experiments an effort has been made to determine the presence of the two types and their proportion to each other in milk produced under different conditions of cleanliness and held for 24 and 48 hours at 60° F. (15.6° C.), 70° F. (21.1° C.), and 86° F. (30° C). The *B. coli* and *B. aerogenes* types were divided on the basis of the methyl-red test proposed by Clark and Lubs (3). The results of the work as shown in Table 15 give the total and colon counts, the number of *B. coli* and *B. aerogenes* types in fresh milk, and the colon count and the percentage of the two types in the same milk held at different temperatures.

Table 15 shows that, as a rule, the colon count of fresh milk comprised only organisms of the *B. coli* type. This statement is based on the results obtained when 1/10 and 1/100 of a cubic centimeter of milk were plated. In a few cases the colonies from the fresh milk were of the *B. aerogenes* type. After the milk was held for a time and further examined both types of organisms were found in most cases. It must be remembered that even when the figures indicate that all the organisms of the colon-aerogenes group were of one type it does not mean that none of the other type were present. This is true because the method of differentiation was the picking off as near as possible of all the colonies of the group which appeared on the plates, inoculating them in a dextrose-peptone broth as described by Clark and Lubs (3), and determining their reaction by methyl red. It is evident, therefore, that the dilution in plating might have been so high as to exclude entirely the type that was in the minority.

From the results obtained it seems safe to conclude that both types are usually present in raw milk. Since milk usually contains both types, it might be assumed that the proportion of *B. coli* to *B. aerogenes* would be of value in tracing the conditions under which the milk was produced.

TABLE 15.—*Proportion of B. coli and B. aerogenes types in different grades of milk.*

Sample No.	Conditions.	Fresh.		24 hours.									48 hours.					
				60° F. (15.6° C.).			70° F. (21.1° C.).			86° F. (30° C.).			60° F. (15.6° C.).			70° F. (21.1° C.).		
		Total count.	Colon count.	Colon count.	Per cent.		Colon count.	Per cent.		Colon count.	Per cent.		Colon count.	Per cent.		Colon count.	Per cent.	
					B. coli.	*B. aerogenes.*		*B. coli.*	*B. aerogenes.*		*B. coli.*	*B. aerogenes.*		*B. coli.*	*B. aerogenes.*		*B. coli.*	*B. aerogenes.*
1	Certified milk	10,400	[1] 0	110	100	0	27,900	99	1	.....	.....	.....	37,000	.....	.....	12,600,000	.....	.....
2		10,100	[2] 20	280	44	56	79,000	75	25	.....	.....	.....	.....	.....	.....	.....	.....	.....
3		8,200	[1] 0	0	.....	.....	400	100	0	.....	.....	.....	1,900	.....	.....	1,130,000	100	0
4		4,200	[1] 0	140	.....	.....	3,700	.....	.....	.....	.....	.....	62,000	91	9	1,120,000	100	0
5		11,400	[1] 0	0	.....	.....	400	.....	.....	.....	.....	.....	4,600	100	0	5,300,000	100	0
6		10,100	[1] 0	220	.....	.....	9,300	.....	.....	.....	.....	.....	169,000	80	20	11,800,000	98	2
7		22,800	[1] 0	0	.....	.....	700	.....	.....	.....	.....	.....	6,400	84	16	3,700,000	.....	.....
8		7,800	[1] 0	0	.....	.....	6,500	100	0	.....	.....	.....	7,100	.....	.....	7,600,000	.....	.....
9		15,700	[1] 0	150	100	0	5,800	96	4	.....	.....	.....	36,000	46	54	1,510,000	39	61
	Average	.....	.....	.....	.....	.....	.....	94	6	.....	.....	.....	.....	80	20	.....	88	12
1	Barn cleaned daily, utensils sterilized	5,800	100	86,000	98	2	5,400,000	91	9	.....	.....	.....	14,600,000	95	5	91,000,000	100	0
2		10,200	0	58,000	52	48	1,720,000	59	41	.....	.....	.....	3,700,000	67	33	31,000,000	94	6
3		13,400	0	124,000	25	75	5,900,000	56	44	.....	.....	.....	1,750,000	6	94	8,800,000	38	62
4		3,400	100	3,900	17	83	1,480,000	7	93	.....	.....	.....	990,000	7	93	52,000,000	0	100
5		6,800	[2] 100	139,000	5	95	610,000	27	73	.....	.....	.....	2,900,000	10	90	.....	.....	.....
6		3,300	10	3,600	54	46	109,000	77	23	.....	.....	.....	2,900,000	74	26	.....	.....	.....
7		2,800	[2] 10	900	50	50	78,000	87	13	.....	.....	.....	1,180,000	72	28	.....	.....	.....
8		2,100	0	2,400	5	95	14,000	30	70	.....	.....	.....	600,000	2	98	.....	.....	.....
9		3,500	110	69,000	100	0	500,000	99	1	.....	.....	.....	14,700,000	83	17	.....	.....	.....
10		10,300	20	1,100	62	38	123,000	74	26	.....	.....	.....	.....	.....	.....	.....	.....	.....
11		11,200	0	3,200	0	100	5,000	100	0	.....	.....	.....	250,000	50	50	.....	.....	.....
12		18,200	0	400	100	0	28,000	100	0	.....	.....	.....	180,000	60	40	.....	.....	.....
13		15,400	10	1,200	83	17	23,000	96	4	.....	.....	.....	1,030,000	3	97	.....	.....	.....
14		69,000	120	9,200	94	6	1,050,000	96	4	.....	.....	.....	.....	.....	.....	.....	.....	.....
	Average	.....	.....	.....	52	48	.....	72	28	.....	.....	.....	.....	44	56	.....	58	42

1	Barn and cows dirty, utensils not sterilized	207,000	1,800	720,000	100	0	28,200,000	100	0				22,900,000	100	0	470,000.000	97	3
2		640,000	1,500	190,000	61	39	14,200,000	79	21				19,300,000	33	67	49,000,000	88	12
3		510,000	200	199,000	97	3	33,000,000	92	8				26,500,000	94	6	144,000,000	98	2
4		54,000	100	10,400	97	3	850,000	89	11				2,780,000	92	8	113,000,000	95	5
5		344,000	200	87,000	98	2	2,600,000	100	0				18,100,000	97	3	26,900,000	98	2
6		237,000	700	2,800,000	95	5	13,800,000	97	3				36,000,000	93	7	118,000,000		
	Average				91	9		93	7					85	15		95	5
1	Barn cleaned daily, utensils sterilized	5,100	0				206,000	74	26	108,000,000	32	68						
2		8,000	[2] 200				273,000	75	25	37,000,000	100	0						
3		1,100	0				39,000	83	17	20,300,000	96	4						
4		2,400	0				3,400	37	63	1,300,000	8	92				600,000	0	100
5		7,700	0				680,000	90	10	2,600,000	87	13				1,680,000	13	87
	Average							72	28		66	34					7	93
1	Barn and cows dirty, utensils not sterilized	23,900	0				35,000	70	30	8,400,000	100	0						
2		39,400	0				33,000	80	20	3,450,000	100	0						
3		46,000	0				9,600	91	9	6,300,000	100	0				39,000,000	0	100
4		155,000	300				208,000	100	0	290,000,000	100	0				38,000,000	65	35
5		38,000	200				930,000	100	0	203,000,000	100	0						
6		46,000	0				23,000	79	21	1,200,000	99	1				850,000	66	34
7		97,000	0				76,000	51	49	870,000	69	31						
8		189,000	0				670,000	97	3	63,000,000	0	100				96,000,000	98	2
9		1,320,000	100				10,800,000	93	7	238,000,000	0	100				162,000,000	100	0
	Average							84	16		74	26					66	34

[1] None in $\frac{1}{10}$ of a cubic centimeter; other "0" in colon count indicates none in $\frac{1}{100}$ of a cubic centimeter.
[2] All *aerogenes* types. When not marked they were all of the *B. coli* type.

The results of the first experiments carried on in connection with this point are shown in sections 2 and 3 of Table 15, where it will be noted that the average percentage of the *B. aerogenes* type was considerably higher in milk produced under clean conditions and handled in sterilized utensils than the percentage found in milk produced under dirty conditions and handled in unsterilized utensils, for any given temperature and time. The average percentage of the *B. aerogenes* type was consistently higher in milk produced under clean conditions when stored at 60° F. (15.6° C.) and 70° F. (21.1° C.) up to a period of 48 hours. Further experiments with milk produced under the same two sets of conditions, but held at 86° F. (30° C.) showed a similar high proportion of *B. aerogenes* in the milk produced under clean conditions, although the actual percentage of the *B. aerogenes* in the milk produced under dirty conditions was also somewhat higher than when held at the lower temperatures. These results are shown in sections 4 and 5 of Table 15.

Further analysis of the results shows that among the samples produced under a given condition there was a wide variation in the proportion of the *B. coli* to the *B. aerogenes* types. Further than this, the proportion of the two types after 24 hours' incubation was often entirely reversed after 48 hours at the same temperature. Considering the higher average percentage for the *B. aerogenes* type in milk produced under clean conditions than under dirty conditions, it seemed that this might be a significant fact in tracing the conditions of production.

The results obtained, however, from a few samples of certified milk as shown in section 1 of Table 15, seemed to indicate that no hard and fast rule can be attached to the proportion of the two types. In view of the earlier findings with milk produced under clean conditions it was thought that certified milk would, upon holding at temperatures above 50° F. (10° C.), show a high percentage of the *B. aerogenes* type, but the result shows that this was not the case.

Based on these results it seems evident that both types of organisms of the colon-aerogenes group are always present in raw milk, but the differentiation into the *B. coli* and the *B. aerogenes* types is of little or no value in indicating the conditions under which the milk was produced or the age and temperature at which it has been held.

The conclusions reached in regard to the value of differentiating the *B. coli* and the *B. aerogenes* types are based on a study of relatively few samples. While the results obtained indicate that the proportion of *B. coli* to *B. aerogenes* types is of little value in determining the history of the milk, it is believed that this point deserves much further study before any more definite conclusion can be reached.

HAS THE COLON COUNT ANY SIGNIFICANCE IN RAW MILK?

Before discussing the question of whether the colon count in raw milk has any significance it must be pointed out that the colon count as made at present includes both *B. coli* and *B. aerogenes* types, of which the *B. coli* type is principally of fecal origin and the *B. aerogenes* type mainly of nonfecal origin. If, therefore, it could be assumed that the number of organisms of the *B. coli* type was a direct measure of the amount of manure in milk (an impossible assumption), the colon count would not even then be a direct measure of such contamination, since it may include the *B. aerogenes* type. Since, however, it is impossible at present to differentiate between the two types by plating methods alone, the significance of the colon count must be considered as it stands, irrespective of the fact that it measures the number of both types of organisms.

The significance of the colon count must be considered in two distinct relations; first, in its connection with fresh milk and, second, in its connection with milk which has been held.

SIGNIFICANCE IN FRESH MILK.

The colon count is, generally speaking, a measure of contamination during production only when fresh milk (less than two hours old) is examined. In fresh milk the colon count measures the degree of contamination from several sources. The organisms gain entrance directly from manure, from unsterilized utensils, and in small numbers from the air and from other minor sources.

Milk produced under clean conditions and handled in sterilized utensils usually does not show the presence of organisms of the colon-aerogenes group in $\frac{1}{10}$ of a cubic centimeter, and probably they are not present in even larger quantities. In milk produced under dirty-barn conditions and handled in unsterilized utensils these organisms can usually be found in $\frac{1}{10}$ of a cubic centimeter and may run as high as 2,000 per cubic centimeter. The colon count of fresh milk therefore bears a somewhat direct relation to the conditions under which the milk was produced. A similar relation, however, does not exist between the colon count and the amount of manurial contamination, first, because different samples of feces vary widely in their colon content, and, secondly, because these organisms may be introduced into milk through other channels, particularly from unsterilized utensils.

SIGNIFICANCE IN MILK THAT HAS BEEN HELD.

When milk is not fresh, no significance can be attached to the colon count in relation to contamination during milking unless it is known that the milk is comparatively fresh and that it has been kept at temperatures not exceeding 50° F. (10° C.). Perhaps a safer limit

would be 45° F. (7.2° C.). In other words, if the history of the milk is not known it is impossible to interpret the colon count in terms of the condition under which it was produced. This situation arises because of the fact that organisms of the colon-aerogenes group grow rapidly in milk when it is held above 50° F. (10° C.).

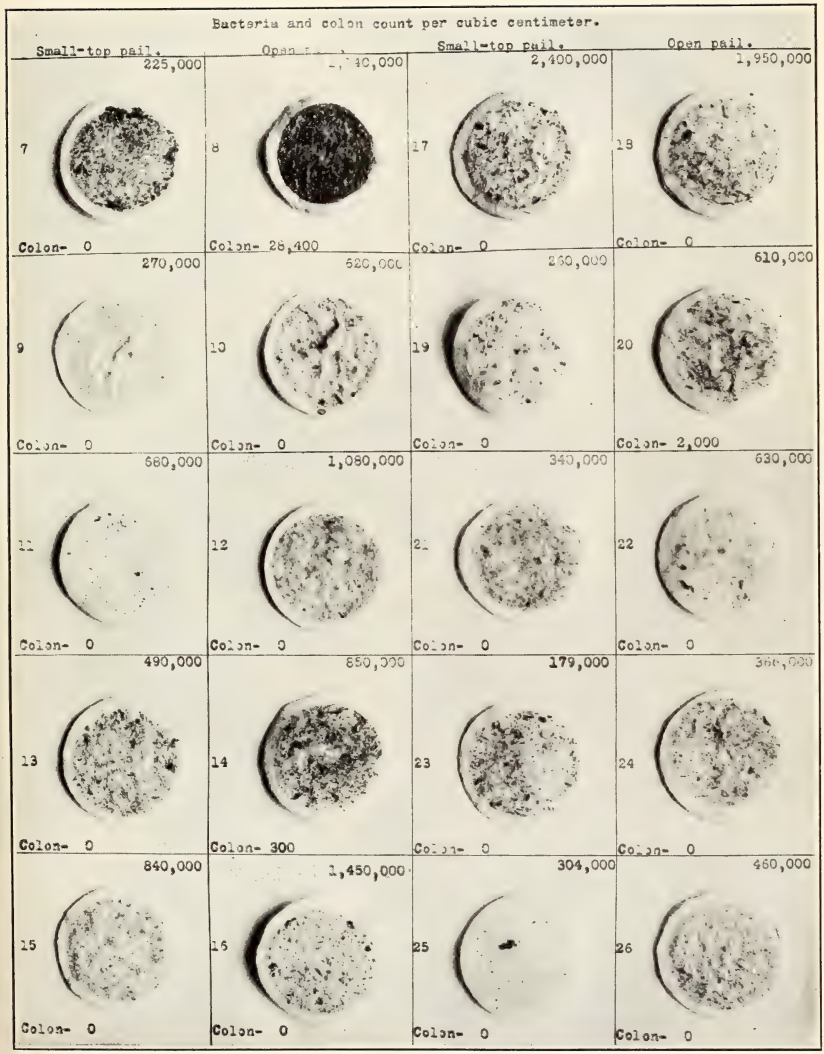
When the history of the milk is not known, it is believed that colon counts above 2,000 per cubic centimeter indicate growth. This belief is based on the fact that only one sample of fresh milk out of the 254 samples which were produced under conditions ranging from clean to extremely filthy contained more than 2,000 colon per cubic centimeter. If, therefore, a sample of milk of unknown history is examined and found to have a colon count between 100 and 2,000 per cubic centimeter, it is impossible to decide whether the colon count indicates original contamination or whether this small number of organisms was due to the growth of the few originally introduced into a very clean milk. Colon counts above 2,000, however, under normal conditions can be attributed to growth. In this connection the colon count is significant so far as it indicates that the milk has at some period in its history been held above 50° F. (10° C.).

There is one abnormal condition of production under which fresh milk may have a high colon count. As previously pointed out, in one series of experiments where utensils were allowed to contain milk drainings for 24 hours during extremely hot weather, and then washed carelessly in lukewarm water without the use of brush or washing powder, the colon count of the fresh milk averaged about 1,000,000 per cubic centimeter. An attempt was made to duplicate these counts under similar conditions, but without success. It is believed that this extreme condition of production would rarely be encountered in the commercial production of milk. High colon counts due to this condition can probably be readily distinguished from similarly high colon counts which are due to growth, because the ratio of total count to colon count would probably be less than 1 to 50 in the fresh milk, while in that due to growth the ratio would be much higher.

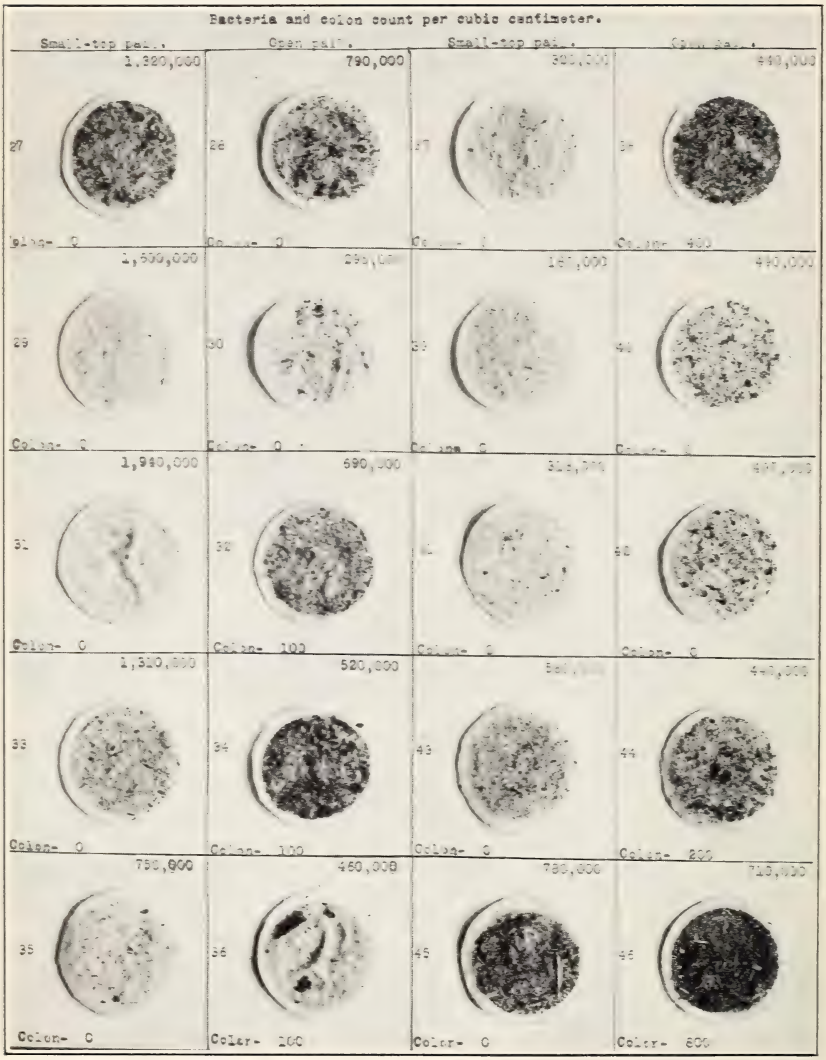
SUMMARY.

1. The colon count as determined at the present time includes both the *B. coli* and *B. aerogenes* types of organisms. Since the *B. coli* type is principally of fecal and the *B. aerogenes* type of nonfecal origin, the colon count at best can not be a direct measure of manurial contamination.

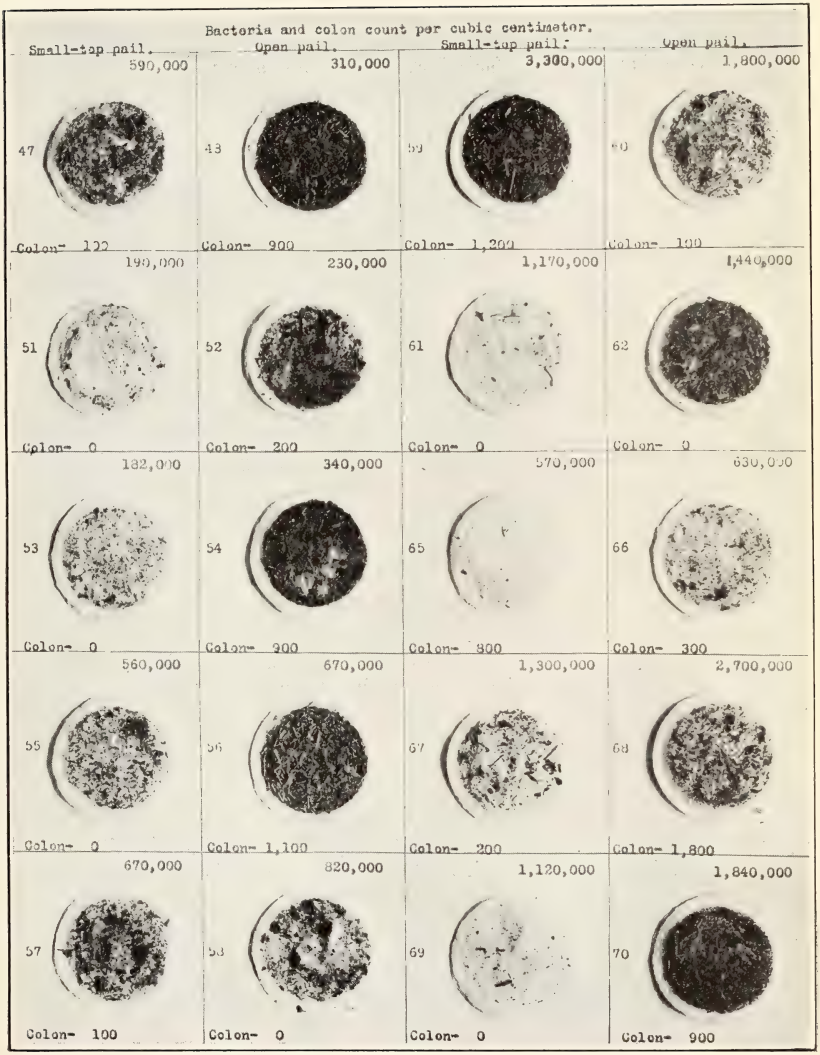
2. Fresh milk produced under the best conditions always contains some organisms of the colon-aerogenes group, but rarely contains over 2,000 per cubic centimeter even when produced under the worst conditions normally encountered.



SEDIMENT DISKS FROM PINTS OF MILK PRODUCED IN DIRTY BARN WITH UTENSILS NOT STERILIZED BUT WASHED AT ONCE AFTER PREVIOUS MILKING.



SEDIMENT DISKS FROM PINTS OF MILK PRODUCED IN DIRTY BARN WITH UTENSILS NOT STERILIZED BUT WASHED AT ONCE AFTER PREVIOUS MILKING.



SEDIMENT DISKS FROM PINTS OF MILK PRODUCED IN A DIRTY BARN WITH UTENSILS NOT STERILIZED AND NOT WASHED UNTIL 8 HOURS AFTER PREVIOUS MILKING.

3. High colon counts can nearly always be attributed to the growth of organisms originally introduced into milk. Little or no growth of the organisms of the colon-aerogenes group occurs below 50° F. (10° C.), but rapid growth takes place at higher temperatures.

4. The differentiation of the *B. coli* and *B. aerogenes* types does not assist materially in tracing the conditions under which milk is produced, but future results from a greater number of samples may show that it is of some value.

5. In fresh milk the colon count does not indicate the extent of direct manurial contamination, but does indicate the general conditions of cleanliness under which the milk was produced.

6. In milk of unknown history no significance can be attached to the colon count other than that high counts usually indicate that it has been held above 50° F. (10° C.), or rarely, that it was produced under very abnormal conditions, as previously described.

7. A careful study of the limitations of the colon count as an index of cleanliness in milk production, taking into account especially the effect of temperature on the growth of the organisms, will materially assist the laboratory man in makin the colon count serve to the best advantage.

LITERATURE CITED.

(1) AYERS, S. H., COOK, L. B., and CLEMMER, P. W.
1918. The four essential factors in the production of milk of low bacterial content. U. S. Dept. Agr., Bul. 642.

(2) AYERS, S. H., and JOHNSON, W. T., JR.
1915. A bacteriological study of retail ice cream. U. S. Dept. Agr., Bul. 303.

(3) CLARK, W. M., and LUBS, H. A.
1915. The differentiation of bacteria of the colon-aerogenes family by the use of indicators. *In* Jour. Infect. Diseases, v. 17, no. 1, p. 160–173.

(4) HARRISON, F. C., SAVAGE, A., and SADLER, W.
1914. The milk supply of Montreal. Macdonald College (McGill University), Ste. Anne de Bellevue, Quebec, Bul. (unnumbered).

(5) HUNTER, O. W.
1917. The colon-aerogenes group from silage. *In* Jour. Bact., v. 2, no. 6, p. 635–639.

(6) JACKSON, D. D.
1908. The determination of intestinal bacteria in milk. *In* Amer. Jour. Pub. Hyg., v. 18, no. 1, p. 16–18.

(7) JOHNSON, B. R., and LEVINE, MAX.
1917. Characteristics of coli-like organisms from the soil. *In* Jour. Bact., v. 2, no. 4, p. 379–401.

(8) KENDALL, A. I., DAY, A. A., and WALKER, A. W.
1914. Studies in bacterial metabolism: 33, The metabolism of the coli-proteus cloacæ group in milk. *In* Jour. Amer. Chem. Soc., v. 36, no. 9, p. 1944–1947.

(9) KINYOUN, J. J.
1914. A bacteriological index for dirt in milk. *In* U. S. Naval Med. Bul., v. 8, no. 3, p. 435–442.

(10) KINYOUN, J. J., and DIETER, L. V.
1912. A bacteriological study of the milk supply of Washington, D. C. *In* Amer. Jour. Pub. Health, v. 2, no. 4, p. 262–274.

(11) KINYOUN, J. J., and DIETER, L. V.
1912. On the preparation of Endo's medium. *In* Amer. Jour. Pub. Health, v. 2, no. 12, p. 979–980.

(12) PRESCOTT, S. C.
1914. Report of hearing on ice cream before Dr. C. L. Alsberg, Chief of the Bureau of Chemistry, U. S. Dept. Agr., Feb. 10 and Mar. 7, 1914, p. 88–119.

(13) RACE, JOSEPH.
1918. The examination of milk for public health purposes. 1st ed.

(14) ROGERS, L. A., CLARK, W. M., and EVANS, A. C.
1914. The characteristics of bacteria of the colon type found in bovine feces. *In* Jour. Infect. Diseases, v. 15, no. 1, p. 100–123.

(15) ROGERS, L. A., CLARK, W. M., and EVANS, A. C.
1915. The characteristics of bacteria of the colon type occurring on grains. *In* Jour. Infect. Diseases, v. 17, no. 1, p. 137–159.

(16) ROGERS, L. A., CLARK, W. M., and EVANS, A. C.
1916. Colon bacteria and streptococci and their significance in milk. *In* Amer. Jour. Pub. Health, v. 6, no. 4, p. 374–380.

(17) SAVAGE, W. G.
1906. Streptococci and leucocytes in milk, 1. *In* Jour. Hyg., v. 6, no. 2, p. 123–138.
(18) SAVAGE, W. C.
1912. Milk and the public health.
(19) SCHROEDER, E. C.
1908. The relation of the tuberculous cow to public health. *In* U. S. Dept. Agr., Bur. Anim. Indus., 25th Ann. Rpt., p. 109–153.

ADDITIONAL COPIES
OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT
10 CENTS PER COPY